

# Genetic Snapshots

## Help Brighten Switchgrass's Future

Elite bioenergy switchgrass growing in eastern Nebraska.

Corn has captured public attention as *the* crop with potential to quench America's thirst for ethanol and other such biofuels. Another fuel-friendly crop is switchgrass. Indeed, ARS-led research in the Midwest indicates an acre of biomass (stems and leaves) from this warm-season perennial grass has the potential to yield 300 to 800 gallons of ethanol.

That's a promising estimate, but more research is needed to improve the conversion technology used and to make the plant biomass easier and less costly to convert into ethanol. Conversion is done by breaking down the plant's cell walls into sugars and then fermenting them.

One approach is to develop new switchgrass varieties with traits geared to producing ethanol rather than traditional uses, like feeding cattle, anchoring soil, or restoring grasslands.

"The ideal switchgrass for bioenergy production would have low input requirements, good stand establishment—especially the first year—high yield, and excellent conversion-to-ethanol properties," comments Gautam Sarath. He's a molecular biologist in ARS's Grain, Forage, and Bioenergy Research Unit (GFBRU), Lincoln, Nebraska.

### Building Living "Libraries"

To expedite breeding efforts, Sarath and collaborators generate tens of thousands of genetic "snapshots" of switchgrass in action—from the moment it sprouts from seed to the time it prepares for overwintering.

The snapshots are actually fragments of genetic material called messenger RNA (mRNA). In plant cells, mRNA delivers instructions for making proteins and carrying out other tasks assigned by DNA—the so-called blueprint for life.

Extracting mRNA from switchgrass offers a glimpse of how this molecular workhorse does the bidding of DNA at particular growth stages or physiological moments in development. In a later step, a technique called "microarray analysis" allows scientists to visually identify which genes were active when they plucked the mRNA from the grass's tissues.

The mRNA is difficult to work with outside its natural setting—cells. So, the researchers create a more stable version—complementary DNA (cDNA). Using standard biotech methods, they insert the cDNA into specially engineered plasmids, which can be propagated in *E. coli* bacteria. Plasmids are circular molecules of DNA found outside chromosomes.

Thus engineered, the bacteria are cultured on plates, where they form thousands of colonies. At this stage, they become known as "libraries," because each bacterial colony contains a plasmid with a unique cDNA from switchgrass.

Since 2003, Sarath and collaborator Paul Twigg of the University of Nebraska-Kearney have produced several cDNA libraries from switchgrass. From these, Christian Tobias, a molecular biologist at ARS's Genomics and Gene Discovery Research Unit in Albany, California, has determined the structure or sequence of some 12,000 previously unknown switchgrass gene fragments.

### Genetic Diamonds in the Rough

In a preliminary analysis of the sequences, Tobias and co-investigators grouped about 65 percent of the new sequences into clusters based on commonalities in their structures. Each of these groups may prove to be a unique gene. The sequence fragments were then compared with databases containing well-characterized genes to provide insight into the possible function of each new switchgrass sequence.

“A closer examination of fragments within clusters revealed that some seemed to have some slight variations. These variations are of interest because they might lead us to a trait that we want to investigate further,” Tobias points out. “These sequence variations reflect and reveal a portion of the genetic variability within the world’s switchgrass gene pool and can be both associated with desirable traits and used in breeding and switchgrass-improvement programs.”

Tobias and Sarath posted the gene sequences to publicly accessible databases on the Internet in 2005. This treasure trove of new discoveries is the most extensive catalog of switchgrass genes yet available for scientists everywhere to use. Researchers can, with the aid of computers, quickly compare and contrast the structure of switchgrass genes to those of other grasses or other forms of life.

Genes from one organism that look like those from another may perform the same job in both. And if that job has already been discovered for the one organism, “you have a head start in correctly identifying its role in switchgrass,” explains Tobias.

Using this comparative approach, Sarath and Twigg have pinpointed a cluster of 12 to 14 genes regulating production and deposition of lignin, a molecular “glue” that binds components of plant cell walls. Sarath notes that bioenergy researchers are keen on weakening lignin’s grip—either through conventional breeding or genetic engineering—to free up more sugars from cell walls for fermenting into ethanol. (See story on page 4.)

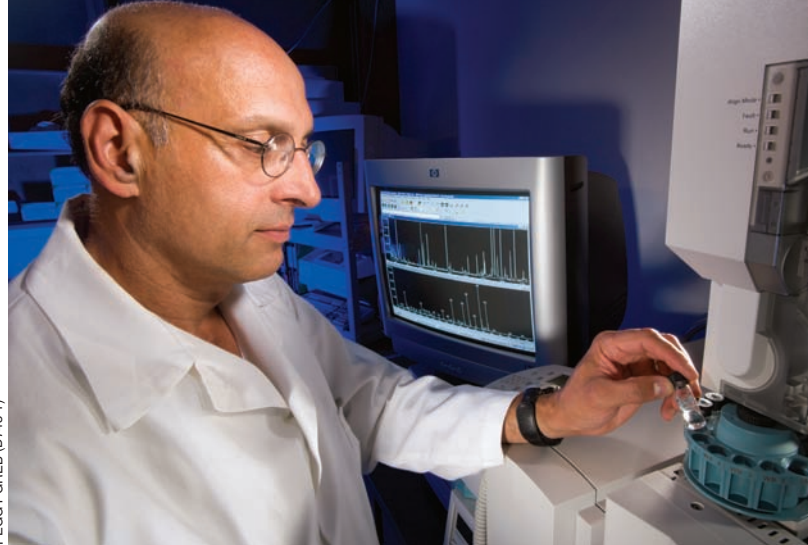
The team’s original cDNA libraries came from a single switchgrass variety. Others will be added, including lowland bioenergy-switchgrass types from a breeding and economic-evaluation program run by GFBRU research leader Kenneth P. Vogel and rangeland scientist Robert Mitchell.

### Helping Hands

The scientists are sending these libraries and RNA to the U.S. Department of Energy’s Joint Genome Institute in Walnut Creek, California. There, fast, state-of-the-art gene-sequencing instruments will identify up to a half-million switchgrass sequence fragments, called “expressed sequence tags” (ESTs), within the next 3 years. These sequences will be compared with those from other plants—particularly other grasses, such as corn and rice—providing invaluable data.

“These ESTs will give us the tools to really understand, or look for, genes important for breeding purposes,” adds Sarath. For this ambitious venture, he and Tobias have already supplied several of the requisite cDNA libraries for the institute’s ultrafast analyses.

Meanwhile, ARS researchers elsewhere are exploring innovative new ways to improve switchgrass for biofuel and other uses. Some examples of this research include:



PEGGY GREB (D745-1)

**Molecular biologist Gautam Sarath loads vials containing hydrolyzed switchgrass cell-wall samples for analyses of lignin content by gas chromatography-mass spectrometry. The data will be used to identify elite switchgrass plants for improvement through breeding.**

- Studying how plant cell walls are made in order to learn how best to break them down—research that should make forage crops like switchgrass more digestible for livestock and more degradable for biofuel production (Hans Jung, dairy scientist, St. Paul, Minnesota).

- Developing switchgrass management tools and conducting greenhouse-gas life-cycle assessments to provide the best combination of biofuel yield and quality, and environmental benefits (Paul R. Adler, agronomist, University Park, Pennsylvania).

- Using enzyme treatments to extract phenolic acids as value-added co-products (Danny E. Akin, microbiologist, Athens, Georgia).

- Putting both dilute acid and enzymes to work in obtaining fermentable sugars from switchgrass and other species (Bruce S. Dien, chemical engineer, Peoria, Illinois).

From seed to fermentable sugars, such research is helping expand switchgrass’s horizons beyond the prairie and into the bioenergy plants of tomorrow.—By **Jan Suszkiw** and **Marcia Wood**, ARS.

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PEGGY GREB (D748-1)



**In Nebraska, technician Marty Schmer harvests switchgrass to evaluate yield potential. These trials will guide breeding and management efforts to increase switchgrass yields.**

## Choosing the Right Switchgrass

Because of its many admirable traits, switchgrass is valued as a robust biomass producer, livestock forage, and restorer of once-pristine prairie lands. Still, many farmers may want to know: Which switchgrass types are best suited for my lands?

Farmers in northern U.S. latitudes may want to check with Michael D. Casler, a plant geneticist with ARS's U.S. Dairy Forage Research Center (DFRC) in Madison, Wisconsin.

After 10 years of switchgrass breeding and evaluation, Casler says he has a pretty good sense of which cultivars for biomass productivity grow best where.

For instance, farmers in southern Minnesota would be wise to choose the cultivar Cave-in-Rock, says Casler, instead of Blackwell, which is better suited to Kansas and Oklahoma.

Making Casler's switchgrass selections even easier is a laboratory test developed by fellow DFRC researcher Paul J. Weimer. With the help of enzyme-rich rumen fluid extracted from the digestive tracts of dairy cows, the method can quickly predict the fermentability, or ethanol-producing potential, of a given cultivar.

Over the next few years, Casler and Weimer will be working to integrate desirable fermentation traits into switchgrass to increase its farmgate value.—By **Erin Peabody**, ARS.

STEPHEN AUSMUS (D762-1)



ARS technicians Christine Odt (left) and Kim Darling dispense rumen fluid into sample vials containing biomass materials during a test to assess the potential of these materials as feedstocks for biofuels production.

WOLFGANG HOFFMANN (D764-1)



Geneticist Michael Casler harvests switchgrass seed as part of a breeding program to develop new cultivars with improved biomass conversion to bioenergy, broad adaptation, and high biomass yields.

## The Gene Pool Deepens

The original cDNA libraries that led to the team's switchgrass gene discoveries came from a variety called Kanlow. As its name implies, Kanlow is best suited for Kansas prairie lowlands. But researchers need to know about the work of genes in the flowers, leaves, stems, and roots of switchgrass plants from throughout its native range.

To make that happen, scientists like ARS plant pathologist Joseph M. Krupinsky at Mandan, North Dakota, have contributed switchgrass tissue to colleague Paul Twigg, to create RNA and cDNA libraries with a richer and more diverse assembly of switchgrass genes. Samples also come from Kenneth Vogel's program at Lincoln.

In all, the new RNA and cDNA libraries are pools or mixtures of specimens, specially selected to add needed breadth, depth, and diversity that otherwise might be missed were the analyses based solely on a single commercial variety such as Kanlow.

The researchers have included individuals encompassing most of the genetic diversity within the 48 conterminous United States.—By **Marcia Wood**, ARS.

